Chapter

Functional Morphology of Gustatory Organs in Caterpillars

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Abstract

The sense of taste plays a pivotal role in the behavior of insects. Caterpillars depend largely on taste cues from plants to detect and locate food sources. Taste stimuli can be either simple or complex as multimolecular mixtures. The insect faces the task of deciphering the nature of these tastants and must then make appropriate feeding choices. Typically, caterpillar larvae possess four types of bilateral gustatory sensilla on their mouthparts. The lateral and medial styloconic sensilla are thought to be the primary organs involved in feeding. These sensilla are in continuous contact with plant sap during feeding and can detect different phytochemicals present in the plant. The gustatory sensory input is encoded as patterns of nerve impulses by gustatory receptor cells housed in these sensilla. Therefore, these gustatory receptor cells form the first layer of a decision-making process that ultimately determines whether food is accepted or rejected by the insect. Caterpillars, such as gypsy moth larvae (*Lymantria dispar*) (L.) (Lepidoptera: Lymantriidae) are major forest pests in most of the United States. These larvae are highly polyphagous feeders and defoliate a variety of tree species, including forest, shade, fruit, and ornamentals. This chapter discusses morphological, feeding behavioral, and electrophysiological aspects of gustatory sensilla with respect to gypsy moth caterpillars.

Keywords: gustation, taste, ultrastructure, insect plant interactions, feeding behavior, electrophysiology

1. Introduction

Gustation is crucial for the survival and nutrition of animals. It is critical in determining the palatability of foods and in providing early warning signs of spoilage. This chapter promotes a better understanding of how natural taste (gustatory) stimuli are recognized, coded, and processed by receptor cells housed in gustatory sensory organs (sensilla) using an insect model, gypsy moth caterpillars, *Lymantria dispar* (L.). These sensilla are cuticular structures which house gustatory receptor cells in them. These receptor cells constitute a sensory filter for environmental taste signals. In insects, these receptors transfer information directly to higher processing taste centers in the brain and form the first layer of a decision-making process which determines if food should be accepted or rejected. Typically, the insect faces the task of deciphering individual tastants in a complex multimolecular mixture to make appropriate feeding choices. In order to respond to stimuli in different behavioral or ecological contexts and to discriminate between meaningful taste stimuli, caterpillars (larvae) have evolved several different types of gustatory sensilla.

Food plant recognition is predominantly governed by the activity of two pairs of sensilla located on the mouthparts, namely the lateral and medial styloconic sensilla [1–3]. When the larva feeds, these gustatory sensilla are in continuous contact with the plant sap and can detect different chemicals (i.e., phytochemicals, secondary plant compounds, allelochemicals) present in the plant. Larval gustatory sensilla provide an excellent system to address questions about the taste system, since: i) these sensilla form a relatively simple sensory system with a limited number of sensory cells that mediate gustatory mechanisms; ii) these sensilla are readily accessible for experimental manipulation, and iii) the receptor cells within these sensilla are individually identifiable and exhibit typically robust and reproducible electrophysiological responses [1].

2. Chemosensory systems and sensillum types

Adult insects possess several different types of sensilla that monitor the environment for cues associated with finding food, oviposition sites, conspecific mates, suitable temperature and humidity levels, and seeking protection and orientation. These sensory organs enable them to detect stimuli associated with taste, smell, touch, sound, vision, proprioception, and geo-, thermo-, and hygroreception. In contrast, the sensory requirements of larvae, such as those found in the order Lepidoptera, are more limited. For example, they rely strongly on gustatory, tactile, and possibly short-range olfactory cues for host-plant selection [4]. Lepidopterous insects use various physical and chemical characteristics to locate plants. Although the visual sense aids a caterpillar to reach a plant, this sense is not finely enough developed to play a role in food plant recognition. The chemical senses, which are well developed in insects, not only guide monophagous insects (feed on only one or a few closely related plant species) to its food, but also helps polyphagous insects (feed on many plants belonging to different plant families) to discriminate various plant species. Chemoreceptors are located on the antennae and mouthparts (**Figure 1**). In total, lepidopterous larvae have five types of bilateral chemosensilla found on the head: a pair of antennae (each innervated by 16 neurons), two pairs of lateral and medial styloconic sensilla located on the galea (each pair innervated by eight neurons), a pair of maxillary palps (each with eight sensilla on their distal surface, and each innervated by 14–19 olfactory and gustatory neurons), and a pair of epipharyngeal organs (each innervated by three gustatory neurons) [1].

Three main categories of insect sensilla exist: (1) AP (aporous) or NP (no-pore) sensilla, which are either mechanosensitive or hygro- and thermosensitive; (2) UP (uniporous) or TP (terminal-pore) sensilla containing gustatory neurons, alone, or with a mechanosensitive cell, and (3) MP (multiporous) or WP (wall pore) sensilla (single-walled (SW) sensilla and double-walled (DW) sensilla). Often, multiporous sensilla are olfactory and wall pore are olfactory and/or thermohygrosensitive [6, 7]. The lateral and medial styloconic sensilla are uniporous or terminal pore sensilla.

3. Lateral and medial styloconic sensilla

The sense of taste in insects is referred to as contact chemoreception. Contact chemosensilla are analogous to the taste buds located on the tongue in the oral cavity of vertebrates. In lepidopterous larvae, gustatory sensilla are located on the mouthparts, specifically the maxillae and epipharynx [6–9]. Each maxilla is comprised of a maxillary palp and galea. Each galea bears two elongated protuberances, namely the lateral and medial styloconic sensilla (**Figures 1** and **2**). These sensilla are located

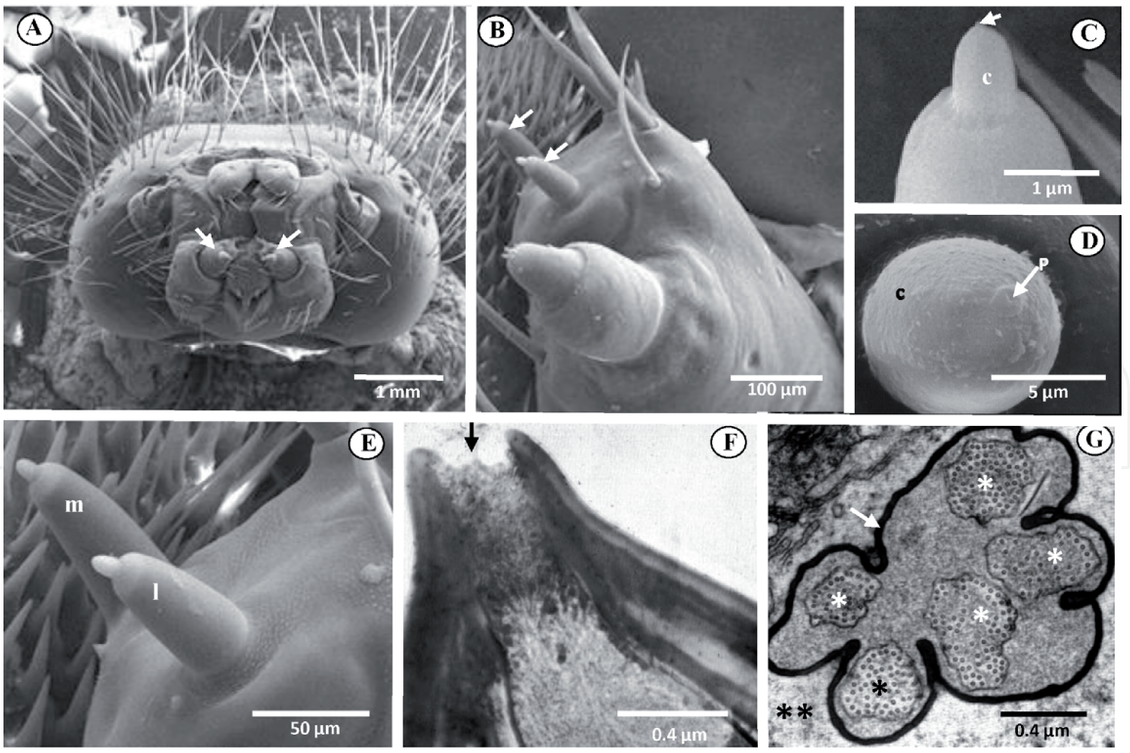

Figure 1.
A-E, scanning electron micrographs and F, G, transmission electron micrographs of Lymantria dispar (L.) *fifth instar larvae. The specimens shown A-E, critical point dried. A) Frontal view, whole head. The arrows point to the galeae, components of the maxillae. Bar = 1 mm. B) Superior-dorsal view of the tip of a left galea showing lateral and medial styloconic sensilla (arrows). Bar = 100 µm. C) Side view of a medial styloconic sensillum showing a higher magnification of a cone (c) inserting into the style (cylindrical projection beneath the cone). The arrow denotes the location of a terminal pore. Bar = 1 µm. D) Higher magnification view of the apical view of a cone (c) showing the terminal pore (p with arrow) from a lateral styloconic sensillum. Bar = 5 µm. E) Higher magnification view of figure B showing the lateral (l) and medial (m) styloconic sensilla. Bar = 50 µm. F) Longitudinal section of a lateral styloconic sensillum showing the tip of the pore (arrow), which contains an apparent plug of fenestrated fibrils. Bar = 0.4 µm. G) Cross section taken near the base of the cone, proximal to where it inserts into a long cylindrical projection (style), and proximal to the site of the tubular body within the mechanosensory dendrite. The five distal dendrites (asterisks) (four chemosensory and one mechanosensory) within the dendritic channel and are surrounded by the conspicuous electron-dense dendritic sheath (arrow) and sensillar sinus (double asterisks). Bar = 0.4 µm. From [5].*

near the mouth opening of the caterpillar. During feeding, these sensilla come into continuous contact with the plant sap before it enters the mouth or buccal cavity and can detect different chemicals present in the plant sap (i.e., allelochemicals) [10]. In lepidopterous larvae, food plant recognition is thought to be primarily mediated by the input from each bilateral pair of styloconic sensilla [1–3, 10–14]. Therefore, they are considered the primary sensory organs involved in feeding [15–18].

Ablation experiments have shown that removal of the styloconic sensilla of the tobacco hornworm, *Manduca sexta,* resulted in widening of its host range [19]. Ablation of the styloconic sensilla in this species permitted it to feed on previously unacceptable plants, thus broadening the range of tolerable plant species of this insect [3]. It was concluded that rejection of plants could be mediated by either the medial or lateral styloconic sensillum and that both sensilla were involved in the rejection behavior to different substances [20, 21]. Thus, these results support the notion that receptor cells present in each styloconic sensillum are involved in selectively mediating the blocking of feeding behavior. Brightfield light microscopic studies, as well as transmission electron microscopy, have revealed that these sensilla each bear a single permeable apical pore (uniporous, UP, or terminal pore, TP sensilla) and are typically innervated by five bipolar neurons, four of which function as putative gustatory receptors and one, as a putative

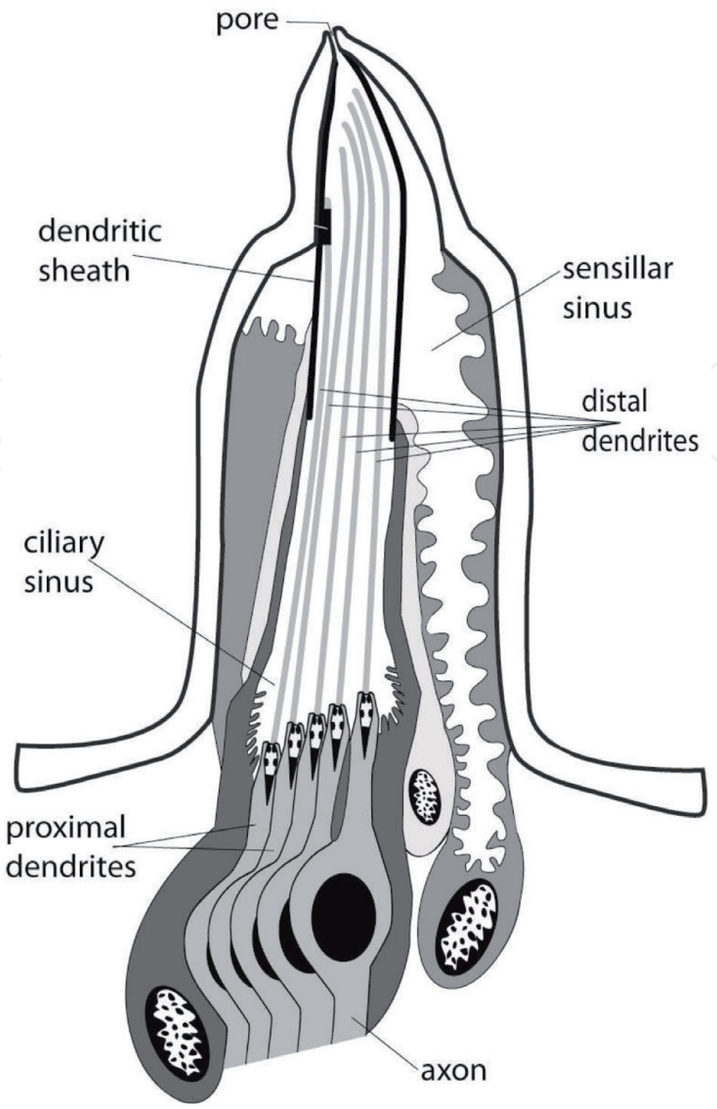

Figure 2.
Diagrammatic reconstruction of a uniporous styloconic sensillum of the gypsy moth, Lymantria dispar*, shown in longitudinal section. Five bipolar neurons are present (four gustatory, one mechanosensory). The dendritic sheath completely separates the dendrites within the dendritic channel from the large sensillar sinus. The sheath ends distal to the small ciliary sinus. In the ciliary region, the distal dendritic segments insert into the proximal dendritic segments. Modified from [9].*

mechanoreceptor [1, 6, 8, 9, 22] (**Figure 2**). The styloconic sensillum is so named, since it appears as a small cone, peg, or knob-like structure that is inserted into a cylindrical projection (style) of insensitive cuticle [23] (**Figures 1** and **2**) and is, therefore, classified as a uniporous (UP) sensillum [6, 7].

4. Structure of uniporous styloconic sensilla

A uniporous styloconic sensillum can take the form of a short to medium-long peg or cone that is inserted into a fibrous cuticular socket of the style, which allows it to flex in this articular region (**Figures 1** and **2**). A schematic reconstruction of a styloconic sensillum is shown in **Figure 2**. The sensillum bears a single permeable apical pore located at the tip and is typically innervated by five bipolar neurons, four of which function as putative gustatory receptors and one, as a putative mechanoreceptor [1, 8, 9, 24]. The pore, about 10–200 nm in diameter, contains typically pore tubules or plugs of fenestrated fibrils [8, 9, 25] allowing chemical communication to occur between the receptor cells and the external environment.

The pore fibrils may also confer selectivity to the conduction mechanism and specificity of response to the sensillum [6, 25]. Four putative gustatory neurons extend within a dendritic channel inside the sensillum from the pore. A dendritic sheath encloses the dendrites. This sheath extends from near the tip of the sensillum to approximately the level of the ciliary sinus. This sinus bathes the dendrites. This sheath completely separates the dendrites from a large sensillar sinus. The dendritic sheath, possibly perforated by pores in some regions, could enable the sensillar sinus to act as reservoir of ions and resting potentials, as has been shown for taste sensilla of adult flies [7, 25, 26]. The fifth putative unbranched mechanosensory dendrite begins near the base of the cone and lies closely apposed to the dendritic sheath and cuticular wall of the cone. The apical termination of this dendrite bears an accumulation of microtubules. These microtubules lie parallel to one another within an electron-dense matrix (tubular body) and is thought to be the site of sensory transduction of mechanical stimuli [27]. The dendrites constrict abruptly midway along their lengths in the ciliary region. This point distinguishes the distal dendritic (ciliary) segments from the proximal dendritic segments. The proximal dendrites continue proximally and form cell bodies. From this point, axons from the lateral and medial styloconic sensilla merge and form the lateral and medial branches of the galeal nerve and project directly without synapsing into the sub-esophageal ganglion (SOG) [28, 29]. The SOG is thought to serve as the first order relay station in the central nervous system. The SOG also exerts motor control over the mouthparts that are directly involved in the feeding process [30–32]. Much of the central processing of various types of input (including gustatory cells) takes place in the SOG, however since inputs from other parts of the central nervous system (e.g., frontal ganglion, olfactory lobes) also contribute to feeding behavior (i.e., host-plant recognition), it is unclear if the "feeding center" is wholly situated in the SOG [33].

5. Feeding behavior

All insects are selective to some extent in their food choice, feeding on (a) one or a few closely related plant species (monophagy), (b) a larger number of hosts usually confined within a certain plant family (oligophagy) or (c) many plants representing a wide taxonomic range (polyphagy). Insects never feed on all plant groups, however [34]. The main function of contact chemoreceptors on the mouthparts of insects is the selection of food. When an insect bites into a plant, some contact chemoreceptors become exposed to the plant sap and function similarly to taste receptors in vertebrates by detecting the compounds in solution [11–14]. However, some mouthpart sensilla, such as would be found in lepidopterous larvae (e.g., found on the maxillary and labial palps), often contact the food before the insect bites. The receptors within these sensilla are sensitive to compounds on the dry surface of a leaf when these sensilla are brought into brief contact with the plant surface. This palpatory behavior serves to: (1) allow the insect to receive a more sustained flow of information from the receptors than would be possible if contact were maintained, since the receptors would become adapted and (2) allow the insect to sample a greater leaf surface than if the sensilla would have remained stationary [35]. The information obtained by palpation, therefore alerts the insect to avoid the intake of noxious compounds and to make feeding decisions more rapidly.

Food selection behavior should be compared to a "key-lock" system where the key represents a receptor activity profile [36]. Only when this profile sufficiently corresponds with an innate standard in a pattern recognition area in the central nervous system is a particular behavioral response triggered. When the

incoming sensory information differs too much from the desired pattern, the food is rejected. The central nervous system (or lock), consisting of the SOG and other brain regions, is tuned to recognize sensory patterns. Those patterns recognized as acceptable will release feeding behavior, while others will result in food rejection. The final decision is thought to be made in the SOG. In the case of a specialist feeder, the incoming sensory pattern would have to match more closely a certain norm set by the central nervous system to trigger feeding activity, whereas in a generalist, many different receptor activity profiles can evoke a feeding response. In order to understand feeding behavior, it is necessary (a) to examine which allelochemicals elicit an acceptance or rejection response and (b) to determine the function and number of taste receptor cells within the styloconic sensilla that are involved in mediating acceptance or rejection of food plants, and (c) to describe how the receptor cells housed in these sensilla encode this taste information and transmit it to the central nervous system to prevent (deter) or elicit feeding behavior.

6. Phytochemicals and hostplant preferences

Phytochemicals include primary and secondary plant metabolites. Secondary plant substances (i.e., allelochemicals) are not universally found in higher plants, but are restricted to certain plant taxa (or occur in those taxa at much higher concentrations than in others) and are of no nutritional significance to insects [37, 38]. Plants produce a wide range of secondary metabolites that act as defense compounds from herbivores, as well as microorganisms. In addition, they can serve as attractants for pollinators. Still others share structural similarities to neurotransmitters [39]. Many secondary metabolites may be cytotoxic as they interfere with biomembranes, cytoskeletal proteins or DNA, and can induce apoptosis [40]. Food specificity can be based solely on the presence or absence of secondary metabolites. In certain plant taxa, these compounds can serve also as "sign" stimuli for some specialized insect species allowing them to unambiguously identify their hostplant, as well as act as effective defensive barriers against non-adapted species [34]. Deterrents (secondary plant substances that inhibit feeding) play important roles in host-plant interactions. It has been postulated that hostplant selection or hostplant acceptability is due to the lack of compounds present that inhibit feeding, whereas rejection of non-hostplants is due to the presence of feeding inhibitors or deterrents. The lack of compounds that inhibit feeding and rejection of non-hostplants is due to the presence of feeding inhibitors or deterrents [41]. The term "allelochemic" was coined and defined as a "non-nutritional chemical" that is produced by an individual of one species (plant) that affects the growth, health, behavior, or population of another species (insects) [37]. Commonly, a plant may produce more than a single allelochemical, which are stored at important sites in the plant [42].

Gypsy moth larvae display a wide host-plant preference [43]. They are highly polyphagous feeders (feed on many plants belonging to different plant families) and defoliate many tree species, including forest, shade, fruit, and ornamentals [44]. For polyphagous ("generalist") insect species, such as the gypsy moth, there may be a balance that exists between phagostimulants and deterrents which determine the extent to which a plant will be eaten or rejected [5, 45–48]. While phagostimulation is necessary to drive feeding, it is not likely to influence hostplant selection [35]. Therefore, hostplant selection is likely defined by the presence of deterrent compounds in non-hosts. Polyphagous insects are deterred

from feeding on plants that store noxious metabolites and usually select those with less active ones [49]. Alternatively, they may also avoid intoxication by changing hostplants rapidly and have evolved detoxification and rapid excretion mechanisms for certain allelochemicals [49, 50]. In contrast, for many oligophagous (feed on several plant species, belonging to the same plant family) and all monophagous (feed on only one or a few closely related plant species) ("specialist") insect species, feeding appears to be driven by the presence of chemicals that act as "sign stimuli." That allow the insect to unambiguously identify their hostplant and stimulate feeding, as well as act as effective defensive barriers against non-adapted species and identify the presence of deterrent compounds in non-host plants [35, 49]. These "sign stimuli" may have been originally noxious but can be tolerated (detoxified) and/or sequestered for the insect's defense against predators or show a relative lack of deterrent effects in the hostplant [35, 49]. Gypsy moth larvae are "generalist" feeders and capable of destroying entire forests during outbreak years. Relatively few studies have documented which allelochemicals are relevant in eliciting acceptance or rejection feeding responses in this generalist herbivore (e.g., [43, 46–48, 51–54]. There is only one study to date that has described the detailed ultrastructural morphology and sensory physiology of chemoreceptors housed within the maxillary galeal styloconic sensilla, thought to be the primary organs involved in feeding [9]. Consequently, our knowledge of the basic mechanisms of chemoreception of gypsy moth larvae lags that of other lepidopterous larvae, such as *Manduca sexta*, *Pieris brassicae*, and *Bombyx mori* (reviewed in [35, 55]. Two-choice feeding behavioral bioassays using *L. dispar* caterpillars revealed that plants containing alkaloids, one of the largest chemically heterogenous groups of allelochemicals, occurring in 20–30% of higher plants, were unfavored by gypsy moth larvae (**Figures 3** and **4**) [43, 46, 47].

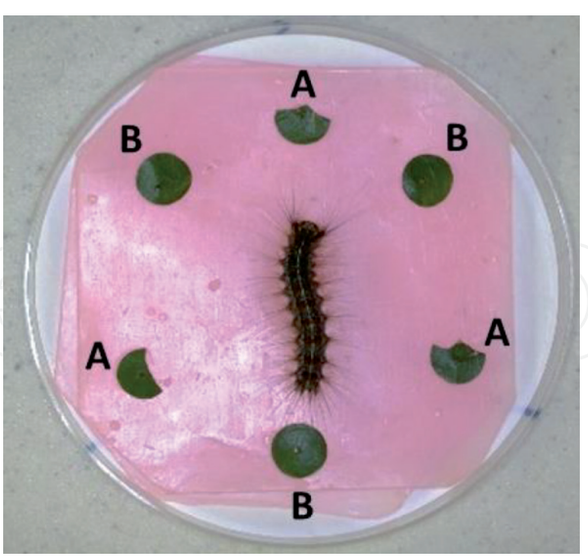

Figure 3.
Experimental set-up for two-choice feeding behavioral bioassay showing the arrangement of control (A) leaf disks and those treated with an alkaloid (B). The disks were punched out of red oak, Quercus rubra (L.)*, a plant species highly favored by* L. dispar *larvae and arranged in an alternating circular fashion (ABABAB) (technique modified after [41]. Metal pins were pushed through the center of each disk into dental wax to ensure that the disks stood ca. 5 mm above the wax surface. The test compounds were dissolved in appropriate solvents and applied so that the chemical amounted to 1% of the dry weight of the disk. Experiments were run until 50% of total area of either control or test disks were consumed. Leaf disks were oven-dried following each experiment for 48 h and then weighed. Values were reported as percent relative mean consumption of control consumption.*

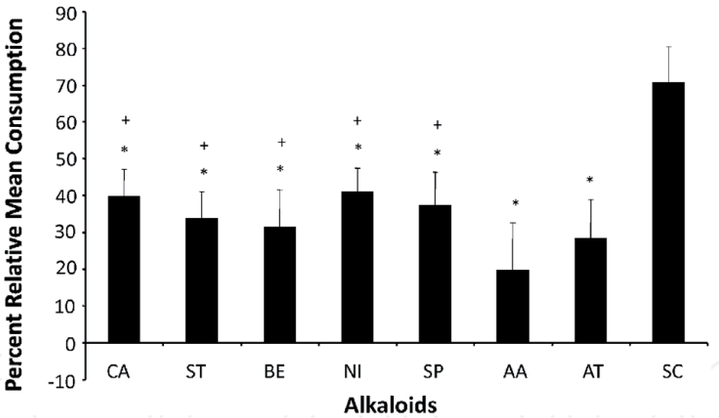

Figure 4.
Two-choice feeding bioassay showing the results of percent relative mean consumption of eight selected alkaloids when applied to red oak leaf disks by fifth instar L. dispar *larvae. Consumption was normalized with respect to control disks (100%). Bars represent the alkaloids tested. AA aristolochic acid, AT atropine, BE berberine, CA caffeine, NI nicotine, SC scopolamine, SP sparteine, ST strychnine. Results are derived from 23, 25, 15, 34, 30, 34, 21, and 15 larvae (number of replicates). Asterisks indicate alkaloids that significantly deterred feeding ($P < 0.05$). Plus symbols indicate alkaloids that were significantly less deterrent on red oak leaves compared with glass fiber disks, i.e., red oak leaves reduce alkaloid deterrent effects. Error bars represent S.E. from [46].*

7. Taste receptor cell classification and peripheral gustatory coding

Insects, like other animals, can taste major nutrients essential for their development, survival, and reproduction, including sugars and inorganic salts. Lepidoptera typically use separate cells that are sensitive to a wide range of chemicals to mediate information about the presence of chemicals, including sugars ("sugar best cell"), inositol ("inositol best cell"), salts ("salt best cell"), and deterrents ("deterrent cell") [38]. In humans, the latter compounds would taste "bitter" [56]. This classification does not necessarily imply that these cells respond only to these groups of chemicals but are more sensitive to them and are likely to be activated by them.

Sensory inputs from food elicit behavioral responses in insects. There is no direct experimental evidence, however, how inputs from taste receptors are integrated in the central nervous system. There appears, however, to be a direct relationship between the amount eaten and the activity of taste receptor cells to different concentrations of a stimulant. Conversely, as the activity of the deterrent cell increases with concentration of the deterrent, the amount eaten declines [55]. It is presumed that these inputs are brought together in the central nervous system in an additive manner and have positive effects. Deterrents, on the other hand, have negative effects on feeding. Insect gustatory receptors transduce the quality and quantity of the complex plant chemistry into a neural code of action potentials. Complex stimuli resulting from e.g., plant saps often evoke spike trains in several receptor cells innervating one or more sensilla. Typically, each cell type (e.g., sugar best cell versus deterrent cells) can be distinguished based on its spike template and temporal firing pattern (**Figures 5**–7) [35, 58]. The frequency and temporal distribution of action potentials in a spike train contains information about the stimulus. The axons project to and converge in the first relay station, the SOG, without intermittent synapses. Unraveling the sensory code occurs by analyzing "input–output" relationships [58, 59]. This can be achieved by stimulating specific sensilla and quantifying electrophysiological recordings of the trains of action potentials (input), as well as quantifying the behavior (output) based on how much food is consumed [35]. Coding is inferred by making correlations between input and output.

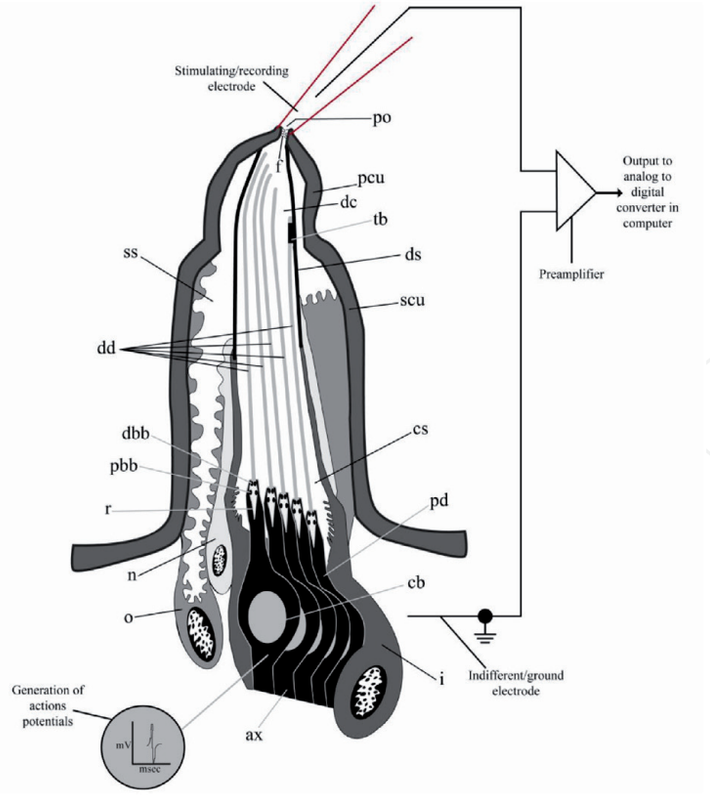

Figure 5.
Diagrammatic reconstruction of a uniporous styloconic sensillum in longitudinal section. This illustration shows, in addition, the electrophysiological tip recording method [57] used to record the excitatory responses from individual taste cells found within a styloconic sensillum. All five sensory cells are shown in this reconstruction. The stimulating or recording electrode contains the taste stimulus dissolved in an electrolyte solution (e.g., 0.1 M KCl dissolved in deionized water). This electrode is placed over the tip and terminal pore of a styloconic sensillum. The solution then diffuses through the terminal pore of the sensillum and taste compounds bind to dendritic taste receptors which transduce the quality and quantity of the taste stimulus into a neural code of action potentials. The other electrode, the indifferent or ground electrode, also contains a similar electrolyte solution and is positioned to make contact with the internal environment of the insect (e.g., body). Both electrodes contain, in addition, a silver wire. The excitatory responses are then recorded, amplified, digitized, and analyzed using a computer software program. Ax, axon; cb, cell body; cs, ciliary sinus; dbb, distal basal body of proximal dendritic segment; dc, dendritic channel; dd, distal dendritic segment; ds, dendritic sheath; f, fibrils; i, inner sheath cell; n, intermediate sheath cell; o, outer sheath cell; pcu, peg cuticle; pd., proximal dendritic segment; po, terminal pore; pbb, proximal basal body of proximal dendritic segment; r, rootlets; scu, style cuticle; ss, sensillar sinus; tb, tubular body. From [5].

To better comprehend the neural communication between chemosensory organs and the central nervous system resulting in acceptance or rejection behavior, three theories exist to best describe the sensory responses: (1) labeled line, (2) across-fiber patterning, and (3) temporal patterning. The first theory proposes that the more important a single compound is in controlling or modifying behavior, the more likely its detection will be coded by a single cell [60]. This "labeled line" (i.e., line or axon along which information is transferred to the brain) to the central nervous system would only carry information from cells with a narrow and well-defined sensitivity spectrum of a specific chemical (or family of chemicals) and would be directly linked to a specific behavioral response [55]. The second theory suggests that the nervous system bases its decision for behavioral output by evaluating the responses from many individual sensory cells with different but overlapping response spectra. The central nervous system extracts meaningful information by reading and processing simultaneous inputs across all afferent sensory fibers (axons) (across-fiber patterning) [61]. This is also known to occur in vertebrates [17]. The third theory implies that temporal patterning may be superimposed on across-fiber patterning suggesting that the ratios of firing across different cells changes with time and can modify a particular

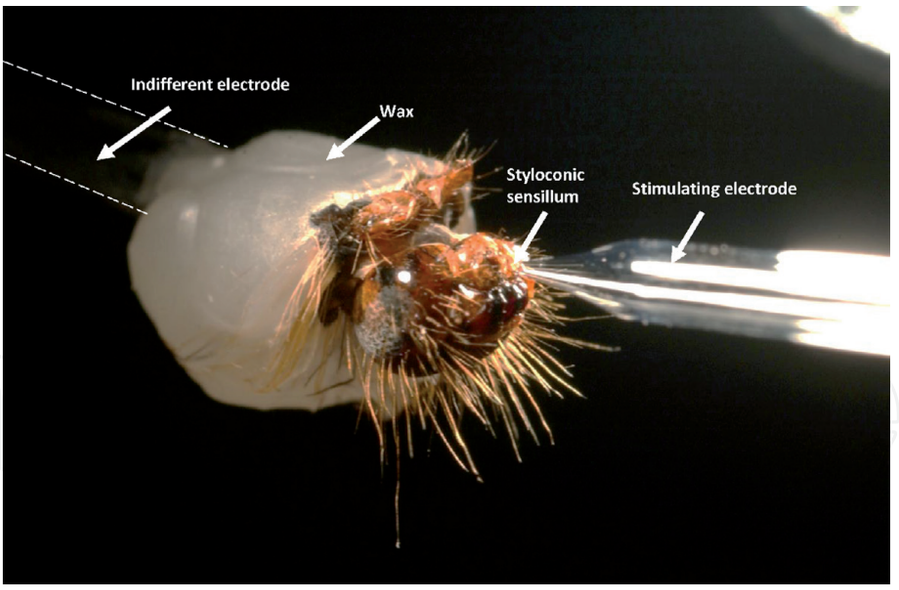

Figure 6.
Photograph of the electrophysiological tip-recording technique as explained in more detail in ***Figure 2****. The stimulating electrode, containing the taste stimulus and dissolved in an electrolyte solution, is placed over the tip of a styloconic sensillum. The indifferent electrode, containing a similar electrolyte solution, is inserted into the body of the insect. A minimal amount of melted wax is used to secure the preparation. From [5].*

message [62]. Most importantly, it should be noted that all three theories (code types) are not mutually exclusive and can be combined into one model [63].

Sensory codes mediating acceptance can: (i) stimulate specific sugar cells coding for an acceptance profile; (ii) stimulate broad spectrum sugar cells that the CNS recognizes as an acceptance profile [62, 64] and (iii) inhibit specific phagodeterrent receptors; this contributes to the neural coding of acceptance [65]. Feeding deterrents may alter sensory input by: (i) stimulating specific deterrent receptors; (ii) stimulating broad spectrum receptors; (iii) stimulating some cells and inhibiting others, thereby changing complex and subtle codes; (iv) inhibiting specific phagostimulant receptors; this contributes to the neural coding of deterrence, and (v) evoking highly unnatural impulse patterns, often at high frequency [65]. The ability of a deterrent neuron to respond to a wide range of chemicals is due to it having a diverse range of receptor sites, each with its own structure–function specificity, or due to the active chemicals having common features making them able to interact with a single receptor site [66]. Deterrent cells possess a number of unique characteristics: (i) they generally adapt more slowly than cells responding to phagostimulatory compounds; (ii) the tonic activity of the deterrent receptor stabilizes at a higher level than in other cell types; (iii) there may be a relatively long latency period prior to the tonic response; (iv) there may be a slow increase in spike frequency following stimulus application, and (v) there may be an increase in spike amplitude with stimulus concentration [62, 67]. Differential adaptation rates are, thus, useful in explaining how a sensory code changes with time and how deterrent receptor activity gradually becomes more pronounced when the sensory message is sent to the brain [62]. Food, which at the beginning of a meal may be acceptable, soon becomes unacceptable because of the more prominent share of the deterrent in the total sensory impression. Using *Pieris brassicae* as a model, it was determined, that impulses from receptor cells that convey deterrent information are given a greater weight by the CNS [55]. Therefore, one impulse from a deterrent-sensitive neuron may neutralize 2.5 impulses from sugar sensitive cells. Furthermore, cells signaling the presence of allelochemicals usually respond to about 1000 times lower concentrations than the receptors measuring the quantity of nutrients.

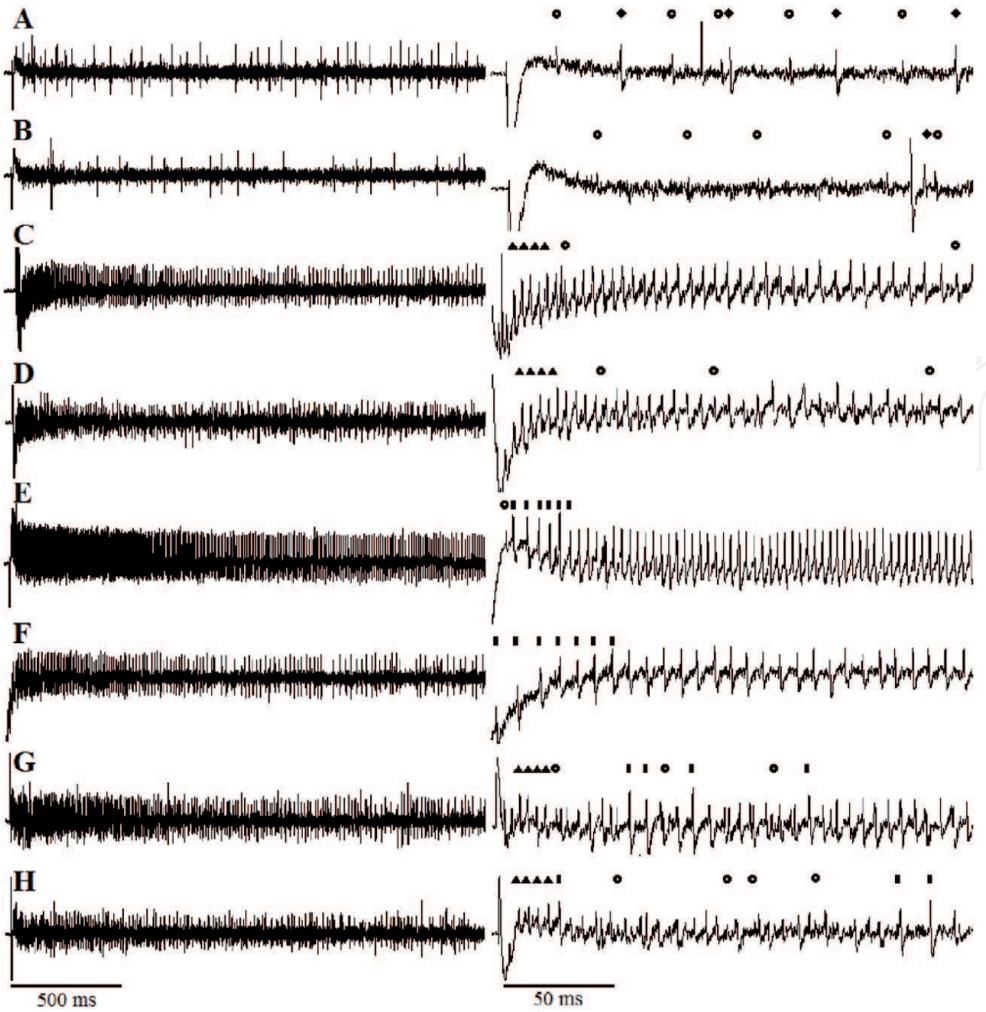

Figure 7.
Representative neurophysiological responses from medial styloconic sensilla of Lymantria dispar *in response to single-component taste stimuli, as well as binary mixtures. The recordings on the left side show only the first 2 s of stimulation. The recordings on the right side are identical to those on the left side but show only the first 200 ms of stimulation. All recordings were made using the same animal preparation. Response of a medial styloconic sensillum to A) 30 mM potassium chloride (salt), B) 100 mM sucrose (sugar) in 30 mM potassium chloride, C) 100 mM inositol (sugar alcohol) in 30 mM potassium chloride, D) the binary mixture of both sucrose and inositol in 30 mM potassium chloride, E) 1 mM strychnine (alkaloid) in 30 mM potassium chloride, F) the binary mixture of 1 mM strychine and 100 mM sucrose in 30 mM potassium chloride, G) the binary mixture of 1 mM strychnine and 100 mM inositol in 30 mM potassium chloride, and H) the mixture of 1 mM strychnine, 100 mM sucrose, and 100 mM inositol in 30 mM potassium chloride. The open circles and filled diamonds represent the firing of the two salt-sensitive cells; the filled triangles, the inositol-sensitive cell, and the filled rectangles, the deterrent-sensitive cell. In A), two taste cells (a smaller and a taller amplitude cell) fired independently of one another in response to potassium chloride. The appearance of a possible third cell in the recording on the right (spike not denoted by an open circle or filled diamond) is the result of both salt-sensitive cells firing at the same time. In B), a sucrose-sensitive cell was absent in medial styloconic sensilla, so only two cells fired in response to potassium chloride, like the response in a. the appearance of a possible third cell in the recording on the right (spike not denoted by an open circle or filled diamond) is the result of both salt-sensitive cells firing at the same time. In C), an inositol-sensitive cell fired in response to inositol. In D), the binary mixture of sucrose and inositol elicited the response of only the inositol-sensitive cell to inositol. The firing rate and amplitude of the inositol-sensitive cell was decreased with the addition of sucrose, implying a mixture-interaction effect. In E) a deterrent-sensitive cell fired large amplitude spikes in response to strychnine. In F), the binary mixture of strychnine and sucrose elicited the response of only the deterrent-sensitive cell in response to strychnine. The firing rate and amplitude of the deterrent-sensitive cell was decreased with the addition of sucrose, implying a mixture-interaction effect and that sucrose ameliorated the deterrent effect of strychnine. In G), the binary mixture of strychnine of inositol elicited the responses of two cells: The deterrent-sensitive cell and the inositol-sensitive cell. The firing rate and amplitude of the deterrent-sensitive cell was decreased with the addition of inositol, implying a mixture-interaction effect and that inositol ameliorated the deterrent effect of strychnine. In H) the mixture of strychnine, sucrose, and inositol elicited the responses of two cells: The deterrent-sensitive cell and the inositol-sensitive cell. The firing rate and amplitude of both cells was decreased with the addition of sucrose, implying a mixture-interaction effect. The addition of both sucrose and inositol ameliorated the deterrent effect of strychnine. From [5].*

8. Conclusions

Insects make ideal models for addressing the mechanisms that govern feeding behavior. As mentioned previously, the gustatory sensilla of lepidopterous caterpillars provide an excellent system to address questions about the taste system. These sensilla form a i) relatively simple sensory system with a small number of sensory cells that mediate gustatory mechanisms, ii) the location of these sensilla provides relatively easy access for experimental manipulation, and iii) the receptor cells within these sensilla are individually identifiable and exhibit typically reproducible electrophysiological responses. The anatomical organization and the molecular signaling pathways in taste are distinctly different between vertebrates and invertebrates (i.e., insects). Nevertheless, in both animal groups, the coding of taste quality has revealed surprising similarities, such that each of the taste qualities is mediated by a labeled line [68]. This means that a particular population of taste receptor cells is set apart and is responsible for encoding a specific taste quality.

At the molecular level, recent research with *Bombyx mori* has revealed that three putative bitter insect gustatory receptors (GRs) (BMGr16, BmGr18, and BmGr53) respond widely to structurally different and partially overlapping deterrents, suggesting that these bitter GRs are feeding deterrent receptors and play important roles in hostplant recognition [69]. Interestingly, feeding preference studies with *B. mori* have shown that the GR66 gene, encoding a putative GR, is responsible for the feeding preference on mulberry of this monophagous insect. With the aid of clustered regularly interspaced short palindromic repeats (CRISPR/CRISPR-associated protein-9-nuclease (Cas9) system, a mutation was introduced in the GR66 locus. As a result of this genetic mutation, *B. mori* larvae broadened their feeding activity. The larvae fed on several plant species not normally in their diet, leading to the discovery of the first genetic and phenotypic evidence that a single bitter GR can affect this insect's feeding preference [70]. The recent progress in functional genomics and molecular advances on bitter GRs of *B. mori*, points to new directions and strategies for controlling pest damage. Furthermore, it broadens our understanding about insect-plant interactions and yields new information about how insects perceive and process taste information.

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